Folate appended chitosan nanoparticles augment the stability, bioavailability and efficacy of insulin in diabetic rats following oral administration

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1. Preparation of folate conjugated chitosan nanoparticles (FA-Ch-NPs)

Activated folic acid (Folate-NHS) was used for surface functionalization and to confirm whether the FA remains stable over the surface, NPs were prepared by using FA-NHS and the pellet of NPs was repeatedly washed (5 times) with PBS 7.4 and further subjected to FTIR spectroscopy (Figure S1).
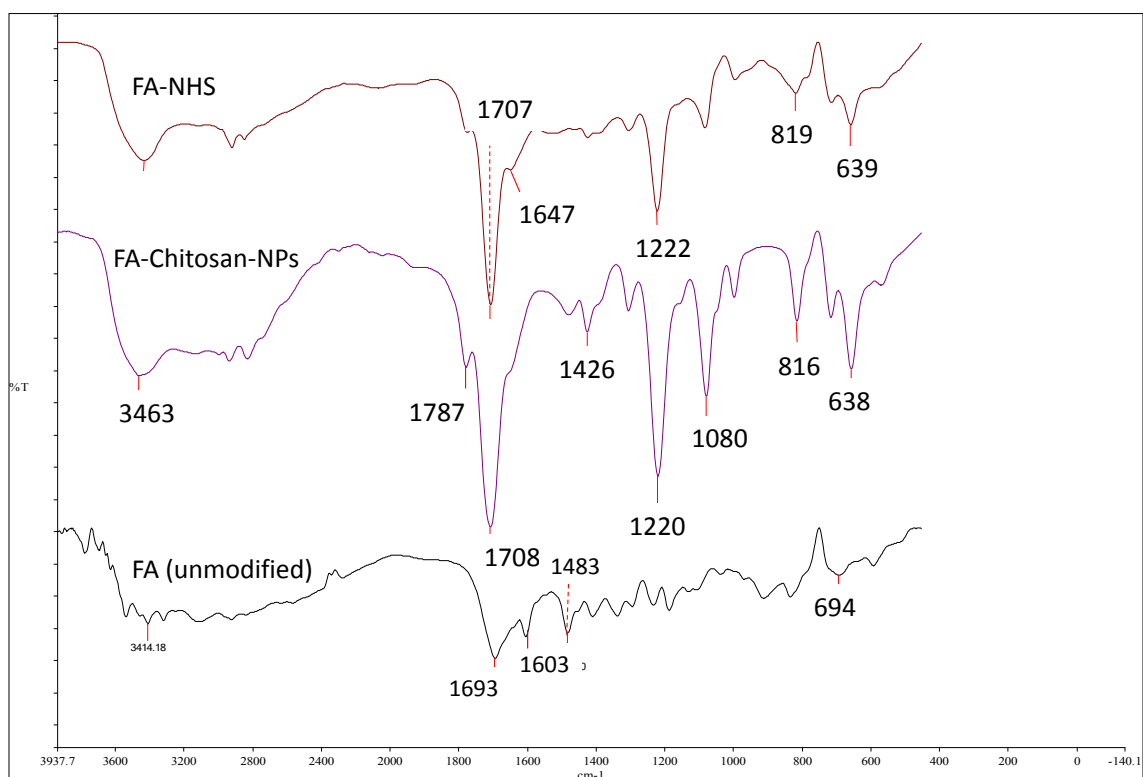
Figure S1: Overlay FTIR spectra depicting the individual spectrum of FA, FA-NHS, Ch-NPs and FA-Ch-NPs formulated by using simple FA as well as FA-NHS

Characteristic peaks representative of the amide I (v **CO)** and carboxyl stretching (v **CO**OH) vibrations, native to the structure of FA were observed at 1603 and 1693 cm^{-1}. After activation a sharp vibration band was observed at 1707 cm^{-1} which was also observed in the FA-Ch-NPs formulated by using FA-NHS even after repeated washing which confirmed the physical stability of surface functionalization using activated FA.

Table S1: Observations used for the optimization of chitosan nanoparticles by using central composite response surface design

Run	pH	PSS	Stirring Speed	Drug Loading
1.	5	0.01	600	20
2.	5	0.01	1800	5
3.	5	0.1	600	5
4.	5	0.1	1800	20
5.	5.75	0.055	1200	12.5
6.	5.75	0.055	1200	12.5
7.	6.5	0.01	600	5
8.	6.5	0.01	1800	20
9.	6.5	0.1	600	20
10.	6.5	0.1	1800	5
11.	5	0.01	600	5
12.	5	0.01	1800	20
13.	5	0.1	600	20
14.	5	0.1	1800	5
15.	5.75	0.055	1200	12.5
16.	5.75	0.055	1200	12.5
17.	6.5	0.01	600	20
18.	6.5	0.01	1800	5
19.	6.5	0.1	600	5
20.	6.5	0.1	1800	20
21.	4.25	0.055	1200	12.5
22.	5.75	0	1200	12.5
23.	5.75	0.055	0	12.5
24.	5.75	0.055	1200	0
25.	5.75	0.055	1200	12.5
26.	5.75	0.055	1200	12.5
27.	5.75	0.055	1200	27.5
28.	5.75	0.055	2400	12.5
29.	5.75	0.145	1200	12.5
30.	7.25	0.055	1200	12.5